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Resistant starch and *in vitro* starch digestibility of five Nigerian foods

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ABSTRACT

There is increased awareness of the role of digestible and resistant starch in the management of some metabolic diseases. The study is aimed at determining the resistant and *in vitro* starch digestibility content of five Nigerian foods. The diets are boiled Cowpeas, *Akara* (fried cowpea cakes), *Moi-moi* (steamed cowpea cakes), *Usi* (gelatinized cassava starch) and *Okpa* (steamed Bambara groundnut cakes). Raw food materials were purchased and processed into commonly eaten forms. Analysis of result showed that *Usi* had significantly ($P< 0.05$) higher total starch content than boiled cowpeas, *Akara, Moi-Moi* and *Okpa*. Rapidly digestible starch was also significantly higher in *Usi* than the other food samples. *Okpa* had significantly lower resistant starch content than other foods. The results suggest that the high rapidly digestible starch content of *Usi* makes it unsuitable for the management of metabolic diseases.

Keywords: resistant starch, *in vitro* starch digestion, rapidly digestible starch.

INTRODUCTION

Starches can be classified based on *in vitro* enzymatic activity. Rapidly digestible starch (RDS) is hydrolyzed to its constituent glucose units after 20 minutes of enzymatic digestion while slowly digestible starch that is converted to glucose after 100 minutes of enzymatic digestion (Sajilata *et al*., 2006). Resistant starch (RS) is also an important measurement to characterize starch digestibility.

Resistant starch (RS) was first recognized although not identified as such in the 1980s when it was found to be resistant to enzymatic hydrolysis (Englyst *et al*., 1992). It was later shown in human ileosteomates that RS bypassed the small intestine and reached the colon where it was fermented to short chain fatty acids (Goldring, 2004). Resistant starch has been defined as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals' (Asp, 1992). Resistant starch has been classified into three major types. The three main forms of RS are: (1) starch that is physically inaccessible to digestive enzymes owing to enclosure in food structures such as intact cells or partly milled or whole grains or seeds (RS 1); (2) resistant starch 2 (RS 2) are ungelatinized starch granules which occurs in raw potatoes and green bananas; and resistant starch 3 (RS 3) are retrograded starch formed during processing of foods (Englyst and Cummings 1990; Englyst and kingman 1990).

Resistant starch in foods affects a number of physiological functions and it has been suggested to have different effects on health. Some documented health benefits include reduced energy content, hypocholesterolemic actions and protective effects against colorectal cancer (Champ *et al*., 2003). Studies on the effect of resistant content of foods on glucose response have been conflicting. Some studies have associated reduction of the glycemic and insulinemic response to food with increased resistant starch content of foods (Behall and Hallfrish, 2002; Robertson *et al*., 2003; Park *et al*., 2004), while others have reported no effect on glucose levels (Sáyago-Ayerdi *et al.,* 2011).

The digestibility of starches has been shown to also affect health. Slow digestion and absorption of carbohydrates has been shown to help maintain steady blood glucose insulin and increase satiety (FAO, 1998; Bornet *et al.*, 2007). There have been studies on the *in vitro* starch digestibility of Bambara groundnut and cowpeas; however there is little or no study on the digestibility of *Okpa*, *Usi, Akara* and *Moi-moi.* Furthermore, there are no scientific data on the resistant starch content of local diets in Nigeria. The aim of the study is to

determine the resistant starch and starch digestibility of five commonly eaten diets namely *Usi, Okpa, Akara*, boiled beans and *Moi-moi*

Materials and Methods

Preparation of Food Samples

Cassava and Bambara groundnut and brown beans purchased from Edjeovyanre Market in Oghara, and Nsukka Market in Delta and Enugu states. The food samples were processed by a trained cook into commonly eaten forms. The processing methods are summarized in the Table 1.

Table. 1 Processing and preparation of Food Samples Osagie and Eka, 1998; Onimawo *et al.*, 2007,

Agricultural forms	Pre- processing	Processing methods
Usi	Cassava tubers were peeled, grinded, filtered and allowed to sediment	Sediment mixed with water and palm oil and heated while stirring in a saucepan till it gelatinzes
Okpa	Bambara groundnut seeds were oven dried at 60^{oc}, de hulled and grinded	Flour mixed with water, tomatoes, pepper, salt, palm oil, seasoning added and paste is steamed in plastic cups.
Akara	Beans seeds were de hulled and grinded	Flour mixed with water, tomatoes, pepper, salt, palm oil, seasoning added and paste fried in vegetable oil.
Boiled cowpeas	No pre processing	Seeds boiled, tomatoes, pepper, salt, palm oil, seasoning added.
Moi-moi	Beans seeds were de hulled and grinded	Tomatoes, pepper, salt, vegetable oil , seasoning added and paste is steamed in plastic cups

In vitro Starch Digestibility

In vitro starch digestibility was determined by modified method according to Holm *et al*., (1988)

Suspensions of samples (100mg/ml) were heated under gentle agitation at 100^{o}C and heated at 100^{o}C. After twenty minutes, the samples were cooled to room temperature and thereafter diluted to 50mg/ml. Porcine pancreatic α-amylase (27mg protein /ml 1200 units/mg) was diluted to 1:200 and 1:400 (a concentration of 200units/g starch where 1 unit liberates 1.0mg maltose from soluble starch in three minutes at pH 6.9 and at 20^{o}C) before incubation . Forty five milliliters (45 ml) of 0.05 mol/L Na-K phosphate buffer (pH 6.9) and 1.25ml of diluted α-amylase were added to samples containing 500mg starch. Samples were analysed after 2hours at 37^{o}C and analyzed with dinitrosalicyclic acid for content of reducing sugar. A standard curve was prepared with maltose. The extent of hydrolysis (the proportion of starch degraded to maltose or percent maltose equivalents times 0.95 divided by milligrams of starch in sample was calculated.

Determination of Resistant and Total Starch

Resistant starch, digestible starch and total starch content were determined according to the method by Mcleary and Monaghan (2002) using a commercially available kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland).

Statistical Analysis

The results were expressed as mean ± SEM (n = number of samples). One-way Analysis of Variance (ANOVA) was used to test for differences among all the groups. Significant differences between groups were detected in ANOVA using Turkey's post hoc test and a value of $p<0.05$ was considered as statistically significant. All statistical analyses were performed using the GraphPad Instat 3 software (GraphPad Software Inc.San Diego, USA).

Results

Table 2 Resistant Starch Content of Raw and Cooked Forms of *Usi* and *Okpa*

Food Samples	Total Starch g/100g	Resistant starch g/100g
Usi	85.02± 2.00^{bcd}	7.56± 0.23^{b}
Okpa	54.75± 2.35^{a}	5.30± 0.78^{a}
Akara	60.31±0.25^{a}	8.34± 0.15^{b}
Boiled cowpeas	69.61±0.45^{abc}	8.55±0.15^{b}
Moi-Moi	63.63 ±0.45^{ab}	6.36±0.30

a indicates a significant different between Usi and other samples, b indicates a significant difference between okpa and other samples; c indicates a significant between akara and other samples; d indicates a significant difference between boiled beans and other samples; e indicates a significant difference between moi-moi and other samples

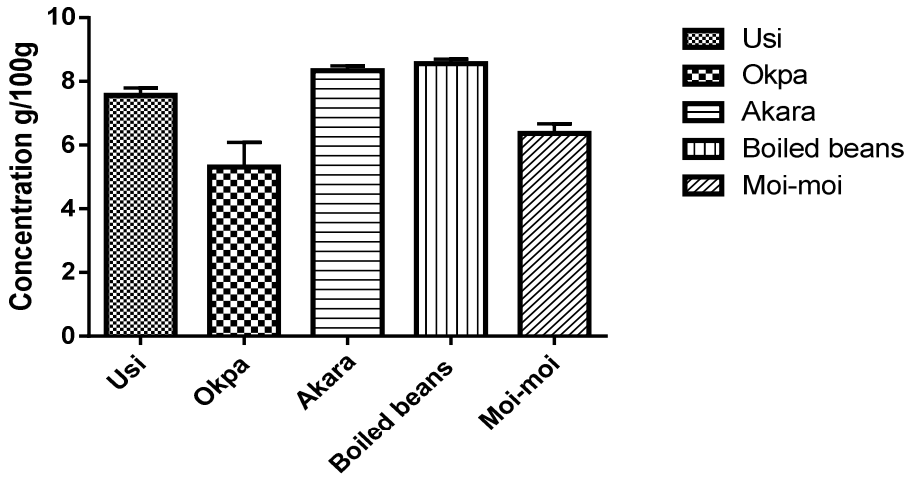

Fig. 1 Resistant starch content of food samples

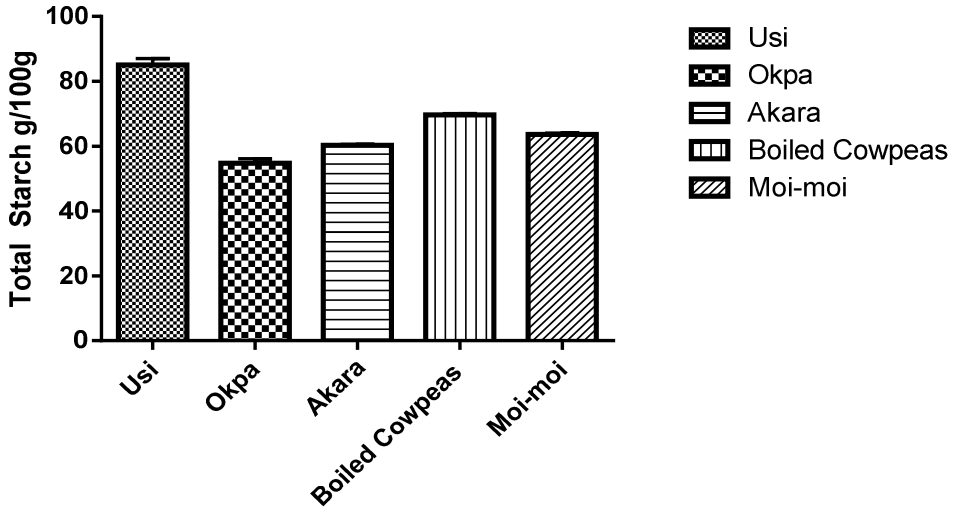

Fig. 2 Total Starch content of food samples

Table 3. *In vitro* starch Digestibility of food samples

Food samples	*In vitro* starch digestibility mg maltose/g sample	% Hydrolysis
Usi	46.79 ± 0.48^{bcde}	93.58
Okpa	43.75 ± 0.02^{acde}	87.5
Akara	26.8 ± 0.41^{abde}	88.89
Boiled Cowpeas	22.59 ± 0.10^{abce}	64.90
Moi-moi	18.60 ± 0.46^{abcd}	54.46

Analyses were carried out in triplicates and values expressed as mean± SEM.

a indicates a significant different between Usi and other samples, b indicates a significant difference between okpa and other samples; c indicates a significant between akara and other samples; d indicates a significant difference between boiled beans and other samples; e indicates a significant difference between moi-moi and other samples.

Table 2 and (Fig 1 and 2) shows the total and resistant starch content of foods. *Usi* (Cassava starch) has the highest starch content while other foods sample moderately high starch content. A significant difference in total starch content ($p< 0.05$) was observed between the total starch content of *Usi* and the other food samples. The lowest total starch is observed in *Okpa* sample. Values of total starch content *Okpa*, Boiled beans and *Moi-moi* were significantly different. A significant difference in total starch content was also observed between akara and moi-moi diets. The results of the resistant starch content indicated in Table 2 show a significant reduction *Okpa* and *Usi,* boiled beans and *Akara*.

The highest starch digestibility was observed in *Usi* as there was 93.58% hydrolysis of starch while 87.5, 88.89. 64.9 and 54.46 was observed in *Okpa*, *Akara* beans and *moi-moi* as shown in Table 3.

Discussion

Usi, also known as "starch", is the local name for a form of processed starch commonly consumed in Delta State. As the name implies, it's not surprising that the results of the total starch content is high. A similar finding was previously reported by Omoregie and Osagie (2011). One of the metabolic consequences of a low fibre, high carbohydrate foods is increased risk of the development of insulin resistance and diabetes (Schulze and Hu, 2005).

The other foods are processed from leguminous plant (either beans or Bambara groundnut), have moderate carbohydrate content; this results agrees with reports by Onimawo *et al*., (2007); Okonkwo and Opara (2010),

Resistant starch content of foods are difficult to find in the scientific literature (Slavin, 2013), there is dearth of data on the resistant content of local diets in Nigeria thus the data on the resistant starch content of foods is novel. The lowest values of resistant content were observed in *Okpa* and *Moi-moi* which have similar processing methods. This suggests that the processing method of these foods may affect the resistant starch content of foods. Removal of seed coats has been reported to reduce the RS1 content of foods (Englyst and Cummings 1990; Englyst and kingman 1990), this may account for the significant reduction in the resistant starch of steamed cakes (*Moi-Moi and Okpa*) and the other foods analysed. The highest resistant starch content was observed in boiled beans; it supports reports by Niba (2003) which shows that boiling increases resistant starch content of some foods. Furthermore, Noah *et al*., (1998) suggests that RS3 may be produced after cooking. *Usi* has a high resistant starch content which may be attributed to its processing method. The processing method employed involves heating the raw starch in a saucepan till it is completely gelatinized and then cooled; this could lead to the formation of Resistant Starch (RS) 3 (Topping et al., 2003; Sajilata et al., 2006).

Usi has a high rate of hydrolysis (93.58%) this shows that it is easily digested. It may be attributed to the high content of amylopectin and the gelatinization of cassava starch. Increased digestibility infers more susceptibility to pancreatic α-amylase indicating that the highly ordered structure of carbohydrates in the food samples has been destroyed to different extent (Holm *et.al*., 1988). Alonso *et al.,* (2000) also suggested that the rupture of starch granules from heat treatment facilitates amylolysis by reducing presence of polyphenols and amylase inhibitors. The difference in amylose to amylopectin content of starches in foods may cause differences in digestibility of starches. Another factor affecting digestibility is the formation of carbohydrate to lipid

complexes. Bennion and Scheule, (2000) suggests that digestibility was further reduced by the addition of oil suggesting the formation of carbohydrate-lipid complexes.

Conclusion

The results suggest that the rate of starch digestibility of *Usi* makes it unsuitable for the management of metabolic diseases.

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